

MALARIA RESEARCH & REFERENCE REAGENT RESOURCE CENTER

Anopheles Rearing Schedule

(Good for many anophelines including *stephensi*, *gambiae*, & *albimanus*. Assumes water temperature of 27°C. Larval development time is longer for *quadrimaculatus* and *freeborni*.)

Schedule 1	Monday	Tuesday	Wednesday	Thursday	Friday Blood	Saturday	Sunday Egg
Schedule 2	Blood	X	x	Egg	Rem/Refeed	Hatch	x
Schedule 1	Monday Rem/Refeed	Tuesday Hatch	Wednesday ×	Thursday split/thin	Friday ×	Saturday split/thin	Sunday ×
Schedule 2	split/thin	X	split/thin	feed	feed	feed	feed
Schedule 1 Schedule 2	Monday feed pupation	Tuesday feed pupation	Wednesday feed pupation	Thursday pupation pupation	Friday pupation x	Saturday pupation x	Sunday pupation x
Schedule 1	Monday	Tuesday	Wednesday	Thursday	Friday Blood	Saturday	Sunday
Schedule 2	Blood			Egg	Rem/Refeed	Hatch	x
Schedule 2 is refeeding of							

Starting a three-week cycle on Friday

<u>Friday:</u> Blood-feed adult females. These should be a minimum of two days old. Practically, wait about 2 days to feed after the last pupae are placed in the cage. You'll notice that the next two days will be conveniently work-free. Think weekend. Place a very well-saturated sugar-water pad on the top of the cup and no wetting will be required over the weekend if the humidity is >80%. During the week, wet the sugar pads on Mon., Wed. and Friday with deionized water. Hereafter, change ALL sugar pads every Friday regardless of condition.

Saturday: No attention required.

Sunday: No attention required.

Monday: Isolate gravid females for isofemale lines (families) or otherwise collect eggs.

<u>Tuesday:</u> Remove the egg dish or remove females from isolation vials. If you have a large number of embryos, bleach the eggs and place in pans for hatching. I place pans directly on the shelf with water, then wash the eggs in without a liner paper. If you don't disturb the pan, no liner is necessary.

<u>Wednesday:</u> Feed hatching larvae 2-3 ml food per pan for mass eggings. Feed families 2 drops per vial.

<u>Thursday:</u> For families, count hatch (optional) and transfer larvae to larger containers. For mass-eggings, no attention is required.

Friday: For families, no attention required.

For mass-eggings, feed, split out into more containers or thin. Do not reuse the hatching pan unless you wipe it with a sponge and rinse it out with hot water. This reduces microbial growth. (Transfer to black containers for color-change induction at this point if necessary.)

Saturday through Wednesday: Continue thinning and feeding pans as needed. Primary indicators for how much to feed: 1. Smell. If you smell a foul odor when you remove the pan cover, you're feeding too much. A healthy organic odor is normal. What is healthy is admittedly a matter of taste J . 2. Turbidity. Yellow water color appears in later stages of rearing (gelbstoff), but if the water is turbid, feed less, or not at all until the water clarifies. 3. Surfactants. When the water is agitated, bubbles that form should burst rapidly. If these persist, filtering the larvae out along with a water change may be in order. If this latter condition occurs, it may be too late to restore the larvae to health.

<u>Thursday through Saturday:</u> Pupae should be collected each day and placed in clean water. Most anophelines have a higher proportion of males the first day so if you are collecting only 100 for stock by the mini-method, check to make sure you have a good number of females. Transfer pupae to emergence cups if you want to examine adults, or pint cups for stocks. Adults require sugar water within about 20 hours after emergence or mortality will occur. If you started the cycle with Friday, pupae should begin to form early enough in the week that you can get adequate numbers for stock before the weekend. If the larvae are crowded or underfed, you'll not get pupae until the weekend.

Friday	of the	following	week.	Blood-feed	the ad	dults to	initiate	the cv	cle again

IF you find that the adults are beginning to die before you blood-feed on Friday, alternate the schedule between a generation of feeding on Monday and then Fridays. This way, every other weekend will be work-free. This makes a 2 1/2 week schedule; better for mosquitoes, not as convenient for mosquito culturist.

Starting a three week cycle on Monday (see above for details)

Monday: Blood-feed adult females.

Tuesday: No attention required.

Wednesday: No attention required.

Thursday: Isolate gravid females for isofemale lines (families) or otherwise collect eggs.

<u>Friday:</u> Remove the eggdish and bleach the eggs if desired or remove females from isolation vials. Change sugar pads.

Saturday: Feed hatching larvae in pans 2-3 ml yeast. For families, feed 2 drops yeast.

Sunday: For mass-eggings, no attention. For families, count hatch (optional) and transfer larvae to larger containers.

Monday: For mass-eggings, feed, split out into more containers or thin. For families, no attention.

Tuesday through Sunday: Thin and feed pans as needed.

<u>Friday through Sunday:</u> Pupae form. If you started the cycle with Monday and have crowded or overfed larvae, pupae will not form until the following Monday.

Monday following week (see note between schedules above): Blood-feed to reinitiate the cycle.